IODINE AS A PARTHENOGENETIC AGENT.

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In the course of some experiments with *Arbacia* eggs at Woods Hole last summer, it was noted that, if a little iodine was added to the eggs in sea-water, regular fertilization membranes appeared on a large number. This led to an attempt to find the optimum conditions for the use of this reagent in parthenogenesis.

The stock solution used was made by dissolving iodine crystals in sea-water to the point of saturation. This stock solution was diluted with sea-water as shown in the tables.

Some of the females were stimulated to shed their eggs into watch glasses. In other cases, the ovaries were removed and shaken in sea-water to loosen them. Whichever method was followed, the eggs were washed and allowed to settle. The supernatant water was poured off, the eggs evenly mixed in fresh sea-water, and 2 c.c. of the mixture put into each of a series of watch glasses. To each of these an equal volume of iodine solution was added, for a given length of time. Some of the eggs were then put into finger-bowls containing about 50 c.c. of sea-water. Others were treated for 20 minutes with hypertonic sea-water (50 c.c. sea-water + 8 c.c. 2.5 N NaCl). At the end of that time, the solution was drawn off and the eggs transferred to finger bowls of sea-water. With each lot of eggs, three controls were made, one fertilized with sperm, one uninseminated, and one uninseminated but treated with hypertonic sea-water.

Table I. shows the percentage of membranes and cleavages produced by varying the strength of the solution and the length of exposure. To obtain these averages, over two hundred eggs were counted in each case.

It will be noted that there is great variability in the results with different batches of eggs. This may be explainable from the fact that the experiments were carried on during the last two weeks of August and the first week of September, a period

TABLE I.

PERCENTAGE OF CLEAVAGES AND MEMBRANES OBTAINED BY TREATING ARBACIA EGGS WITH IODINE, OR IODINE FOLLOWED BY HYPERTONIC SEA-WATER.

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Sea-water.	Not Fertilized. In Hypertonic.	
2.5			14	24											69	3	5	Shed
			14	22											64	2	2	Shaken
	2	7	17	16											68	4	2	Shaken
					26	27	16	16							41	1	4	
					16	32	11	39							69	3	11	
									12	13	12	14			85	7	9	
									5	8	4	12			83	7	9	
5					16	12	14	39	6	16	3	3			44	2	2	Shed
					18	21	30	22	17	17	5	15			32	1	2	Shed
					3	15	20	22	20	23	9	10			83	1	4	Shaken
			1	1	21	2	20	29	14	23					82	5	6	Both
							12	32							3	1	1	Shed
							19	20							94	5	6	Shaken
							16	27							94	5	6	Shaken
									8	21					66	3	5	Both
			20	17											69	3	5	Shed
			19	7											64	2	2	Shaken
	1	1	18	9											68	4	2	Shaken
					49	49	11	5							41	1	4	
					23	37	26	43							69	3	11	
									10	13	9	4			85	7	9	
									2	13	5	10			83	7	9	

TABLE I.—*Continued.*

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.		Controls.			Remarks.
		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.		+ Hypertonic.	Fertilized.	Not Fertilized. In Seawater.	Not Fertilized. In Hypertonic.	
7.5			5	21	29	20	16	13	1	2					13	0	1	Shed
			35	37	28	28	41	31	8	18					70	4	10	Shaken
							21	47							3	1	1	Shed
							30	22							94	5	6	Shaken
							22	27							94	5	6	Shaken
									9	26					66	3	5	Shed
			15	13											69	3	5	Shed
			11	10											64	2	2	Shaken
	7	4	23	16											68	4	2	Shaken
					34	24	24	27							41	1	4	
					25	36	28	27							69	3	11	
10			13	7	23	20	21	19	11	16					88	1	5	Shed
			0	15	30	26	25	19	8	21					51	0	3	Shed
			3	9	37	25	13	33	7	17					88	1	11	Shaken
			3	15	22	16	14	18	11	6					13	0	1	Shed
			28	23	19	23	11	21	23	32					70	4	10	Shaken
			1	1	1	8	26	31	28	28					82	5	6	Both
							45	56							3	1	1	Shed
							19	24							94	5	6	Shaken
							26	37							94	5	6	Shaken
									16	24					66	3	5	Both
			8	5											69	3	5	Shed
			7	14											64	2	2	Shaken
	3	3	11	17											68	4	2	Shaken

TABLE I.—*Continued.*

Time, Min	Sat. Iodine Solution.	+ Hypertonic.	½ Sat. Iodine Solution.	+ Hypertonic.	¼ Sat. Iodine Solution.	+ Hypertonic.	⅛ Sat. Iodine Solution.	+ Hypertonic.	1/16 Sat. Iodine Solution.	+ Hypertonic.	1/32 Sat. Iodine Solution.	+ Hypertonic.	1/64 Sat. Iodine Solution.	+ Hypertonic.	Controls. Fertilized.	Not Fertilized. In Seawater.	Not Fertilized. In Hypertonic.	Remarks.
10					32	44	18	14							41	1	4	
					26	39	18	44							69	3	11	
									15	12	4	15			85	7	9	
									6	17	4	10			83	7	9	
12.5							8	28							3	1	1	Shed
							13	18							94	5	6	Shaken
							23	24							94	5	6	Shaken
									16	26					66	3	5	Both
			12	6											69	3	5	Shed
			7	19											64	2	2	Shaken
	11	3	8	16											68	4	2	Shaken
					18	20	19	46							69	3	11	
15							15	33							3	1	1	Shed
							16	22							94	5	6	Shaken
							17	41							94	5	6	Shaken
									21	35					66	3	5	Both
					24	42	29	29							69	3	11	
									9	11	10	12			85	7	9	
					21	27	7	13					2	5	92	1	2	Shaken
20									15	17	14	15			85	7	9	
					21	24	9	5					2	8	92	1	2	Shaken
25									15	20	8	16			85	7	9	

near the close of the breeding season, when the eggs vary greatly in their fertilizing power and their sensitiveness to reagents. It is probable that the strength found best for this season will prove too great for the more sensitive eggs in the height of the season—July, for instance.

Table II. is a summary of Table I., giving the average number

TABLE II.

SUMMARY OF TABLE I.

Time, Min.	Sat. Iodine Solution.		½ Sat. Iodine Solution.		¼ Sat. Iodine Solution.		⅛ Sat. Iodine Solution.		1/16 Sat. Iodine Solution.		1/32 Sat. Iodine Solution.		1/64 Sat. Iodine Solution.	
		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.		+ Hyp.
2.5	*2*	*7*	15	21	*21*	30	14	28	9	11	8	13		
5	*1*	*1*	15	9	*22*	23	19	27	11	18	6	8		
7.5	*7*	*4*	18	19	*29*	27	26	28	6	15				
10	*3*	*3*	6	12	*24*	25	20	26	14	19	4	13		
12.5	*11*	*3*	9	14	*18*	*20*	16	29	*16*	*26*				
15					*23*	35	17	28	15	23	*10*	*12*	*2*	*5*
20					*21*	*24*	*9*	*5*	*15*	*17*	*14*	*15*	*2*	*8*
25									*15*	*20*	*8*	*16*		

of cleavages plus membranes obtained by each method of treatment. A number in italics is the result of one experiment, rather than the average of several. There is a remarkable uniformity in the percentages obtained by treating the eggs for varying periods of time. For instance, whether exposed for two and one half or fifteen minutes, or for some intermediate period, to one eighth saturated iodine, the eggs gave percentages between 26 and 28. The indication is that the iodine enters the egg immediately and affects it immediately to its full extent. One would expect this result if it acted by combining chemically with something in the egg. While length of exposure has no effect, varying the strength of iodine used causes great variation in the result. This indicates a secondary, non-initiatory or injurious effect due to the excess. The optimum results usually followed treatment of 2 c.c. eggs in sea-water with 2 c.c. of one fourth or one eighth saturated iodine solution. While hypertonic after treatment was nearly always beneficial, in most cases the effect was so slight as to be negligible.

It may be recalled that membrane formation and subsequent

cytolysis were obtained by Loeb,[1] McClendon,[2] and Glaser,[3] in *Arbacia* eggs, by placing them in sea-water diluted with distilled, or in pure distilled water. In the latter case, the eggs became "ghosts" of their former selves, since the pigment dissolved out and increased osmotic pressure caused the egg to absorb water and swell enormously. If an excess of iodine is added to normal eggs, they also become pale, but due to a precipitation of the pigment, which gathers as a small dark mass near one side of the egg. There is seldom indication of swelling or ghost formation.

The membranes obtained by the iodine method were identical with the true fertilization membranes which appear when the sperm enters the egg. They do not resemble the membranes formed by treatment with butyric acid and hypertonic sea-water, which Loeb describes as very difficult to see, excepting by the trained observer. This normal appearance of the membranes was noted by others accustomed to distinguishing types of membrane in *Arbacia*. The significance of the nature of this membrane will be discussed in a later paper.

Reference to Table I. brings out a result that was wholly unexpected. It is commonly supposed that eggs shed by the animal are in better condition and more fertile than those taken from the ovary. The results with one female, whose shed eggs produced only 3 per cent. cleavages, while those shaken from the same ovaries gave 94 per cent., were so striking that the averages were computed. It was found that the shed eggs averaged only 43 per cent. as against 80 per cent. cleavages from the eggs shaken from the ovaries. It is planned to test this during the height of the breeding season, so as to discover whether this is a normal occurrence or whether the "shed" eggs are more likely to be "over-ripe" than the others, late in the season.

[1] Loeb, Jacques, "Die chemische Entwicklungserregung, etc.," Julius Springer, Berlin, 1909.

[2] McClendon, J. F., "On Artificial Parthenogenesis of the Sea-urchin Egg," *Science*, 1909, Vol. 30, pp. 454–455.

[3] Glaser, O. C., "On Inducing Development in the Sea-urchin (*Arbacia punctulata*), Together with Considerations on the Initiatory Effect of Fertilization," *Science*, 1913, Vol. 38, pp. 446–450.